Evaluation of the Intrinsic Acid-Dissociation Constant of Alginic Acid by Considering the Electrostatic Effect

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Alginic acid (AA) is a polyuronide found in seaweeds, such as *Macrocystis pyrifera*, *Laminaria* and *Eklonia* species. The strong affinity of AA to metal ions results in the precipitation of alginates¹, and such a character has been used for the removal of heavy metal ions from waste water.² The metal binding sites in AA are carboxylic groups, which are mainly related to L-guluronic acid.³ Information about the acid-dissociation constant (pK_a) values for the carboxylic groups in AA is necessary to calculate the pH-dependent distribution of metal ions and metal alginate; it is also a useful physicochemical parameter to control the metal binding abilities of AA.

In evaluating pK_a for polyelectrolyte, such as AA, a large electrostatic field was considered; also the intrinsic acid-dissociation constant ($pK_{a,int}$) should be evaluated under an electrostatic-free condition.⁴ Haug determined the pK_a values for AA from two types of seaweeds at an ionic strength of 0.1 M NaCl ($pK_a=3.74$ and 3.42).⁵ However, the $pK_{a,int}$ have not been reported because of a lack of information about the AA polymer. In the present work, while taking electrostatic effect of AA into account, the pK_a values were determined based on the potentiometry at a variety of ionic strengths. In addition, the $pK_{a,int}$ of AA was evaluated by extrapolating the pK_a values to the electrostatic-free condition with an electrostatic model involving the Debye-Hückel theory.

Experimental

The AA was purchased from Nacalai Tesque INC (Kyoto, Japan). To 50 mg of AA in a 100-ml ground-glass stoppered Erlenmeyer flask, 30 ml of water containing inorganic salts was added. The ionic strengths of the solution were adjusted by the addition of NaClO₄, NaCl, NaNO₃ and Na₂SO₄. After shaking for 12 h under a N₂ atmosphere at 25°C, the mixture was placed into a polyethylene titration cell. The titration

was performed under a N₂ atmosphere and the temperature of the cell was maintained at 25±0.1°C. An AUT-501 auto-titration system (TOA Electronics Ltd., Tokyo) was used by connecting with a GST-5311-type glass electrode (TOA Electronics Ltd.). The titrant was a 0.01 M NaOH aqueous solution, which was standardized by using an HCl standard solution (Nacalai Tesque INC). The hydrogen ion activity was corrected to the hydrogen-ion concentration by using the activity coefficient. The total concentration of carboxylic acid in AA (C_{AA}) was determined by a Ba(OH)₂ method.⁶ The concentration of dissociated AA can be written as

$$[AA^-]=[H^+]+C_{NaOH}, \quad (1)$$

where C_{NaOH} denotes the concentration of NaOH in the solution. Therefore, the degree of dissociation (α) was calculated by dividing $[AA^-]$ by C_{AA} .

The molecular weight of AA was determined by gel-permeation chromatography. A 40 µl aliquot of the AA solution (1 g l⁻¹ in 0.1 M phosphate buffer at pH 7) was a Jasco PU-980-type HPLC pumping system (Japan Spectroscopic Co., Ltd.), which was connected to a Shodex RI SE-61-type refractive-index detector (Showa Denko K.K.). An Asahi-Pack GFA-7M column (7.6 mmφ×500 mm, Asahi Kasei) was used as the solid phase; the column temperature was maintained at 50°C. The mobile phase was an aqueous mixture of 0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄ (pH 7.0), and the flow rate was 0.75 ml min⁻¹. The Shodex standard material P-82 (Pullulan) was used as the standard of the molecular weight. The partial specific volume of AA was calculated from the reciprocal of the density, which was determined by measuring the specific gravity at 25±0.1°C according to a method reported by Jones *et al.*⁷

Results and Discussion

Titration curves

Figure 1a shows the pH titration curves of the AA at

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Fig. 1 pH titration of alginic acid in a variety of ionic strength (NaNO_3). (a): acid-base titration curves, (b): modified Henderson-Hasselbalch plots, $n=0.82\pm0.08$.

a variety of ionic strengths (NaNO_3). The acidity of the AA was enhanced with an increase in the ionic strength. This is attributed to the fact that the electrostatic effect of AA decreased with an increase in the ionic strength. It has been known that α can be written by a modified Henderson-Hasselbalch equation⁸,

$$-\log[\text{H}^+] = \text{p}K_a - n \log \frac{1-\alpha}{\alpha}. \quad (2)$$

Equation (2) represents the linear relationships between $-\log[\text{H}^+]$ and $\log\{(1-\alpha)/\alpha\}$. Figure 1b shows a Henderson-Hasselbalch plot, and the apparent $\text{p}K_a$ can be calculated from the intercept of the plot according to Eq.(2). In the present study, the n that was the slope of the plot was in the range of 0.8 – 0.9. These values were smaller than those for a weak acid, such as polyacrylic acid ($n=1.4 - 2.0$).⁸ In polyacrylic acid, the unity of the acidic group is acetic acid, and this $\text{p}K_a$ (4.7) is larger than that for L-guluronic acid (3.7) in AA.⁵ Therefore, the smaller values of n in the AA suggest that AA is a strong acid, rather than a polyacrylic acid.

Evaluation of intrinsic acid-dissociation constant by electrostatic model

As described in Fig. 1 b, the apparent $\text{p}K_a$ values were evaluated by Henderson-Hasselbalch plots at a variety of ionic strength. The intrinsic $\text{p}K_a$ ($\text{p}K_{a,\text{int}}$) can be evaluated under an electrostatic-free condition. The treatment of the electrostatic interaction among the AA molecules was according to a model used for globular proteins, reported by Tanford.⁹ The electrostatic free energy (W_{el}) on the AA molecule is defined by the Debye-Hückel theory,

$$W_{\text{el}} = \frac{Z^2 e^2}{2Dr} \left(1 - \frac{\kappa r}{1 + \kappa a} \right), \quad (3)$$

where D , e , Z , r and a denote the dielectric constant of water, the proton charge, the average charge on the AA molecule, the radius of the AA molecule and the sum of r and the ion radius of H^+ (0.03 nm), respectively. Moreover, κ denotes a Debye parameter (m^{-1}), which is represented as

$$\kappa = \sqrt{\frac{8\pi N e^2 I}{1000 D k T}}, \quad (4)$$

where k , T , N and I denote Boltzmann's constant, the absolute temperature, Avogadro's number and the ionic strength, respectively. Therefore, the κ value at 298 K (25°C) can be written as

$$\kappa = 3.29 \times 10^9 \sqrt{I}. \quad (5)$$

The relationship between the apparent dissociation constant (K_a) and the intrinsic dissociation constant ($K_{a,\text{int}}$) can be written as

$$K_a = K_{a,\text{int}} \exp \left\{ \frac{z Z e^2}{D r k T} \left(1 - \frac{3.29 \times 10^9 r \sqrt{I}}{1 + 3.29 \times 10^9 a \sqrt{I}} \right) \right\}, \quad (6)$$

where z denotes the charge of H^+ (+1). The r was calculated from the molecular weight (M) and the specific partial volume (v). Since the partial molar specific volume is vM , the volume per molecule is vM/N . Therefore, r can be calculated from

$$r = \left(\frac{3vM}{4\pi N} \right)^{1/3}. \quad (7)$$

In the present study, M , which was measured by gel-permeation chromatography, was 63600 ± 680 , and v was $0.963 \pm 0.004 \text{ cm}^3 \text{ g}^{-1}$. Therefore, from Eq.(7), r was calculated to be 2.9 nm.

Figure 2 shows the relationships between $\text{p}K_a$ and the ionic strength in the presence of a variety of anions. The solid lines in Fig. 2 indicate the nonlinear least-squares curve-fittings of the logarithmic form of Eq.(6) to the experimental data points. In Eq.(6), the parameter, Z , indicates the average charge per molecule of AA, which can be calculated from the end point of the titra-

Fig. 2 Relationships between apparent pK_a and $I^{1/2}$ in a variety of anions. The non-linear least square curve-fittings to the logarithmic form of Eq.(5) were represented by solid lines.

Table 1 Intrinsic acid-dissociation constants and average charges on the AA molecule in a variety of anions

Anion	$pK_{a,int}$	$-Z$
ClO_4^-	2.84	64
Cl^-	3.04	50
NO_3^-	3.08	45
SO_4^{2-}	3.43	27

tion. However, these changed with the ionic strength. Although the amount of total carboxylic acid in AA was used to calculate Z , the curve-fittings were poor. When Z was treated as a variable parameter, excellent curve-fittings were obtained, as described in Fig. 2. The variability of Z in a variety of anions may be due to the conformational effect on the AA molecule.

Effect of anions on $pK_{a,int}$ and Z

The $pK_{a,int}$ and Z values, which are evaluated in Fig. 2, are summarized in Table 1. The $pK_{a,int}$ values were in the range of 2.8 – 3.4, and this order was $SO_4^{2-} > NO_3^- = Cl^- > ClO_4^-$. The larger value for SO_4^{2-} is attributed to the fact that SO_4^{2-} behaves as a weak acid, rather than other anions. On the other hand, the order of Z was $ClO_4^- > Cl^- > NO_3^- > SO_4^{2-}$. Because the hydrophobic character increased with decreasing Z , the AA molecule is more hydrophobic in the presence of SO_4^{2-} . In an anionic surfactant, such as dodecyl sulfate, the critical micellar concentration decreased according

to the following order: $SO_4^{2-} > NO_3^- > Cl^-$.¹⁰ This suggests that the conformational changes of polyelectrolyte remarkably occur in the presence of SO_4^{2-} , which brings about a hydrophobic molecular environment. Therefore, we concluded that the changes of $pK_{a,int}$ and Z in a variety of anions are due to a conformational effect as well as the affinity of H^+ to anions.

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